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Short Communication

DETERMINATION OF PROTEIN CONTENT IN VEGETABLE FOODSTUFFS AVAILABLE IN SYLHET BY THE KJELDAHL METHOD

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ABSTRACT

A study was conducted for the estimation of protein in vegetable foodstuffs by the Kjeldahl method. The estimation of protein by the Kjeldahl method is based on the digestion of sample with concentrated sulphuric acid (H_2SO_4) and thus reaction between protein and sulfuric acid resulted in ammonium bisulfate (NH_4HSO_4). On addition of base in the ammonium bisulphate, ammonia gas is evolved which was passed into standard hydrochloric acid (HCl) solution. From the changed strength of HCl, then the solution the amount of nitrogen in the sample and consequently the protein can be estimated. A comparative study of some samples collected in Sylhet and Bhola area was also described.

INTRODUCTION

Proteins are the basis of body structure. They are the building blocks for hair, muscles, skin and many others parts of the body and without protein it would not be possible to replace or repair the cells in body. An ordinary man, weighing 70 kg contain around 11 kg of protein. Proteins are the major organic constituents of protoplasm and many extra cellular substances and are also used to form enzymes, nucleoproteins (nucleic acids, DNA, RNA) and some hormones (Debajyoti D., 1982). Proteins have many different biological functions (Lehninger, A, 1993) and also play a vital role in living organisms as enzymes. Transport proteins such as hemoglobin and myoglobin are of vital importance. The antibodies that are our defense against disease are proteins. Proteins are complex organic compounds which are made of amino acids as the building blocks. Sufficient amount of proteins should be taken so as to maintain the N-balance and to provide for tissue growth and maintenance. The human body requires approximately 20 amino acids for the synthesis of proteins and among 20 only 13 amino acids can be produced in body called non essential amino acids. The remaining 9 amino acid that are obtained from food, and not made in the body. Proteins can be found in animal products such as meat, fish, eggs, miked dairy products, as well as vegetables foods such as: cereals, grains and seeds. Three main types of protein-calorie deficiency diseases such as mrasmus, Kwashiorkor and Marasmic kwashiorkor are often met with in India, Bangladesh and Southeast Asia, West African and Arab countries.

Protein content in biological sources varies from source to source, and even on the place of production of them. Content of protein in different vegetables food-stuffs are different and may vary considerably due to factors which include soil, climate, season. The content of protein in vegetables food-stuffs found in Sylhet area may be different from others because the properties of soil and climate of Sylhet are totally different from that rest of the part of Bangladesh. Therefore, it is important to know is there any variations about the content of protein in different region. So the objective of the present study is to the estimation of protein in different foodstuffs found at Sylhet and to compare the total protein content of some vegetable foodstuff of Sylhet with other regions like Comilla and Bhola.

MATERIALS AND METHODS

There are numerious methods that are employed for estimation of proteins such as Dumas method (Sternanglanz *et al.* 1962), Biuret method (Riegler 1914), phenol-reagent method (Wu 1922), Ninhydrin determination (McGRATH 1972), Graves method (Graves *et al.* 1968), Lowry and modified Lowry method(Peterson 1877; Hartee 1972; Fryer *et al.* 1986, and spectrophotometric method (Warburg *et al.* 1941) etc but most of them have some limitations.

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A calorimetric protein assay technique for the determination of protein concentration was reviewed by Sapan *et al.* (1999). Kjeldahl method nitrogen determination is performed on a variety of substances such as meat, feed, grain and many other samples. Kjeldahl method is widely used for determination of nitrogen and consequently estimation of protein. It is well established method for the determination of protein content in biological food-stuffs and it gives reproducible results. Kjeldahl method has a number of advantages over the classical Dumas method, namely because of its speed and simplicity. Thus considering this advantages and availability of the reagents and micro Kjeldahl distillation apparatus the well established Kjeldahl method have used for the estimation of protein in food samples. Proteinious nitrogen is not only the source of nitrogen in he sample. There are many other sources of non-protein nitrogen. At first the percent of total nitrogen (%TN) and then the percent of non-protein (%NPN) were determined. The percent of protein nitrogen (%PN) was estimated by difference between % NPN and % TN. All nitrogen determinations were estimated by the Kjeldahl method. The true protein was calculated as (total N- non-protein N) x 6.25.
Reagent required: Hydrochloric acid, Sulfuric acid, Trichloroacetic acid. Sodium Sulphate (Na_2SO_4), Copper Sulphate ($CuSO_4$).Sodium hydroxide (NaOH), Sodium carbonate.Methyl Orange etc

Determination of total nitrogen (TN):
Procedure:
The procedure for total nitrogen (TN) determination can be conveniently divided into two parts: (i) Digestion of the sample (ii) Distillation of the digested product.

(i) Digestion of the sample: In a typical procedure 0.5 g sample, 5.0 g sodium sulphate (Na_2SO4) and 0.5 g copper sulphate ($CuSO_4$) with 11 mL of concentrated sulfuric acid (H_2SO_4) was taken in a digestion flask (also called iodine flask). This mixture was heated for about 2 hours until the colour of the digestion mixture changed to colourless. Then the digestion mixture was cooled and about 10 ml of water was added to dissolve the digestion product and to make it ready for the distillation.

(ii) Distillation of the digested product: Digested product obtained in earlier step was poured into the Kjeldahl apparatus with care and approximately 35 ml of sodium hydroxide (40%) was added to that digested product. 20 mL of 1 M hydrochloric acid was taken in a another large capillary tube. After the addition of 35 ml of 40% NaOH solution ammonia gas was evolved and it was absorbed in hydrochloric acid of known concentration. The steam distillation was continued until no ammonia gas is evolved which was tested with red litmus paper and phenolphthalein indicator. After absorption of the ammonia gas, the change of the strength of hydrochloric acid was determined with standard sodium carbonate solution using methyl orange as indicator.

Determination of Non-protein nitrogen (NPN):
Procedure: Approximately 0.2 g sample was taken and was dissolved in 20 ml of water and 30 ml of 20% trichloroacetic acid was added with it. This solution was filtered on a filter paper and the filtrate was subjected to for digestion process. Approximately 5.0 g of sodium sulfate (Na_2SO_4), 0.5 g copper sulfate ($CuSO_4$) and 8.0 ml concentrated sulfuric acid (H_2SO_4) was taken with the filtrate in a digestion flask. The mixture was digested and distilled in the same way as mentioned earlier and the quantity of ammonia as well as the NPN was determined from the change of concentration of HCl solution as mentioned for total nitrogen determination.

RESULTS AND DISCUSSION

Table 1 summarizes the %protein in different samples. From the Table 1 it is seen that the protein content in the same food-stuffs of different places are not so much different. The samples “khesari” (27.26%, entry 4) and “Mogh” (23.52%, entry 6) of Bhola is better than that of Sylhet (“khesari” 26.47% and “Mogh” 22.81%, entry 3,5). On the other hand the sample “Musuri” (20.81%, entry 1) of Sylhet is better than that of Bhola (Mususri” 19.24%, entry 2). So, we can concluded that the soil of one area is not suitable for all types of crops. The animal source of protein, such as fish contain 16% to 25% of protein and meat contain 18% to 25% proteins. Now, from this study it is obvious that, for some vegetable sources, the protein contents are comparable with that of animal sources. Thus we can also say that some vegetable sources are very good sources of protein for human being those are cheaper compared to animal protein. Among the samples studied, the maximum percent of protein was found in “Khesari” (27.26%, entry 4). Thus we can concluded that, vegetable sources of protein like “Khesari”, Mogh , Musuri, Sola, motor etc are excellent sources for the poor people.

CONCLUSION:

Kjeldahl is well established procedure for the determination of protein in biological food-stuff and gives reproducible results. Among the samples studied, khesari is relatively rich (27.26%) in protein than other samples. From the study it is obvious that, for some vegetable protein sources, the protein contents are comparable with that of animal sources. Therefore, we can say that some vegetable sources are very good sources of protein for human being and are cheaper compared to animal protein.

Table 1. Percent protein in different food-stuffs samples

Name of samples	%Total protein N (%TN)	%Non-protein N (%NPN)	%Protein
Musuri (S)	4.2435	0.9130	20.81
Musuri (B)	3.7189	0.6393	19.24
Musuri (C)	3.69	0.62	19.18
Khesari (S)	5.5746	1.3384	26.47
Khesari (B)	6.1588	1.7958	27.26
Khesari (C)	6.1700	1.80	27.31
Mogh (S)	4.8231	1.7958	22.81
Mogh (B)	5.0384	1.2737	23.52
Mogh (C)	4.9200	1.2512	22.93
Rice (boil) (S)	2.5425	0.7585	11.15
Rice (boil)(B)	2.6542	0.8420	11.32
Rice (boil)(C)	2.6342	0.8400	11.21
Rice (non-boil) (S)	1.8974	0.4563	8.39
Rice (non-boil) (B)	1.7749	0.4563	8.24
Rice (non-boil) (C)	1.8110	0.4553	8.47
Motor (S)	4.6559	1.0584	22.48
Sola(S)	4.4998	1.1022	21.23
Flour(S)	1.8193	0.6217	7.48

Note: %Protein=6.25 ×(%TP-%TNP); S = Sylhet, B = Bhola and C = Comilla

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